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COMPARATIVE STUDIES OF SMILAX, SECTION SMILAX, OF THE SOUTHEASTERN UNITED STATES

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The species of the genus *Smilax* L. of the section Smilax (LILIACEAE) are unusual monocotyledons. In addition to possessing reticulate veined leaves, the taxa of this section are woody, dioecious vines with "stipular" tendrils. The combination of the above vegetative characters defines the Smilax section of *Smilax* as a distinctive, easily recognizable group. However, identification of specimens, especially sterile ones, to taxa within the section is often difficult. Also concepts of phylogenetic relationships between taxa are not clearly established and certain leaf characteristics pointed out by Duncan (1967) are not identified as to their structural nature. Therefore, attempting to alter these situations, we investigated each woody species of *Smilax* indigenous to the southeastern United States (Duncan, 1967), except *S. pumila* Walt., for epidermal and gross anatomical similarities and differences of leaves.

The specimens examined in this study are of *S. auriculata* Walt., *S. bona-nox* L., *S. glauca* Walt., *S. laurifolia* L., *S. rotundifolia* L., *S. smallii* Morong, *S. tamnoides* L., and *S. walteri* Pursh. as interpreted by Duncan (1967). Three leaf forms of *S. bona-nox* (auriculate, ovate, and cordate), and two of *S. tamnoides* (orbicular and hastate, varieties *hispida* and *tamnoides*, respectively) were treated separately, making eleven kinds of *Smilax* studied.

Confusion as to the delimitation of the species of *Smilax* dates back at least to 1753 with Linnaeus' *Species Plantarum*. As Fernald (1944) adeptly points out, Linnaeus had no clear understanding of American species, and his citations cover different species. Other investigators have also disagreed regarding taxa. Morong (1894) describes *S. smallii* as a new species while maintaining *S. lanceolata* L. as a separate taxon and recognizes *S. hispida* Muhl. and *S. beyrichii* Kunth *in sensu S. tamnoides* L. and *S. auriculata* Walt., respectively. Small (1933) assigns *S. pseudochina* L. and *S. tamnoides* to the species *S. bona-nox* and *S. beyrichii*, and *S. smallii* to the species *S. lanceolata*. He accepts *S. hispida* as *S. tamnoides*.

Caponetti and Quimby (1956) studied anatomical structures of roots, leaves, aerial stems, and rhizomes of *S. tamnoides*, *S. auriculata*, *S. bona-nox*, and *S. glauca*. They report the last species as the only one possessing papillae on the lower leaf epidermis and having thick-walled circular lignified cells in the region of the mesophyll beneath the midvein. They isolate *S. bona-nox* on the basis of the cells surrounding the midvein having thick lignified walls toward the midvein and thin non-lignified walls away from the midvein.

Arber (1920) studied the anatomy of the tendrils of *Smilax* and interprets them as being equivalent in morphological value to the petiole and having originated through that structure. She maintains that the blade of *Smilax* is not equivalent to the lamina of a dicotyledon, although quite similar in appearance internally, but is merely a "pseudo-lamina" representing an expansion of the upper region of the petiole. The thickened tip which characterizes the blade of some members of the genus is possibly the last relic of the unexpanded petiolar apex. Thus each tendril on her interpretation is equivalent to the petiole and these to the pseudo-lamina.

Coker (1944) made an extensive study of woody *Smilax* of the United States. He gives comprehensive descriptions and discussions of each species, pointing out important

omissions and errors found in manuals and other writings. New data concerning underground parts are emphasized. Information is given on epidermal features of leaves of some taxa, but it is scanty, and mostly concerns stomates.

Sixty-one fresh and dried specimens were utilized in this study. Identification by collection data and University of Georgia Herbarium acquisition number is given by Yates (1968). Two mature leaves were taken for study from each fresh collection, one large and one small. The remainder of the collection was pressed, dried, mounted, and deposited as a voucher specimen. Additional material was obtained for study by removing one mature leaf from each of a number of selected herbarium specimens previously on deposit and reconstituting it by treatment with 0.1 Normal NaOH at 60°C for 24 hours.

An outline drawing was made of each leaf chosen for study and a segment one mm wide cut out one-tenth of the distance of the total length beneath the tip. Two more segments were taken directly below the first one. The leaf segments were placed in FAA (5 ml formalin: 5 ml glacial acetic acid: 90 ml 50% ethyl alcohol).

Epidermal imprints were made from each leaf using a modification of the technique described by Sinclair and Dunn (1961). They were taken along the horizontal axis of the leaf from midvein to margin at a point approximately one-third the total leaf length from the tip. Surface feature studies were first attempted from imprints made from dried material. Our stomatal counts were obtained from these preparations. Later, material preserved in FAA or reconstituted with NaOH was used to make imprints for analyzing the other surface features. Imprints were made from three leaves of each of five collections from each of the eleven kinds of *Smilax*.

The terminology used to describe epidermal cells and venation is self-explanatory; however, those most likely to be misinterpreted will be described below. The others are described by Yates (1968). Veinal cells refer to those epidermal cells in areas above or below veins. Midvein and

lateral veins are those arising at the base of the blade and extending longitudinally to the apex. Secondary veins are those extending horizontally from any one of the above veins to any other. Venule reticulum includes all the small veins in the leaf forming the anastomosing network characteristic of the genus *Smilax*.

For the sake of brevity, the imprints of features of the epidermal cells will be referred to henceforth by the name of the feature involved. Configuration of lateral cell walls is described as straight, curved, or undulate. Straight denotes those epidermal cells having at least three walls without undulations although the walls may be bowed, especially at junctions with adjacent cells. Undulate refers to those cells having at least two walls each with 2.5 or more undulations that are 25 or more microns high. Curved indicates those epidermal cells which fall between straight and undulate.

Microscopic counts and length and width measurements of the stomates were made in five fields of vision 0.093 mm^2 in area on imprints from three dried leaves of each collection. If any part of a stomate was in the field of vision, it was included in the count. The means of these three characteristics were tested for significant differences by Duncan's new multiple-range test (Steel and Torrie, 1960).

For gross anatomical observations, the first segment cut from the large leaf in the procedure described previously was dehydrated through a tertiary butyl alcohol series, embedded in paraffin, and sectioned at ten microns. The sections were mounted using a modification of Haupt's adhesive and stained with Conat's quadruple stain (Johansen, 1940). A second series of sections was stained using aniline blue and safranin as a countercheck on the former. The characteristics of the cuticle, epidermis, mesophyll, and vascular system were studied from these preparations. Two to five specimens of each of the eleven kinds of *Smilax* were studied anatomically.

Freehand cross sections of dried, preserved, and reconstituted material of selected species were stained with a

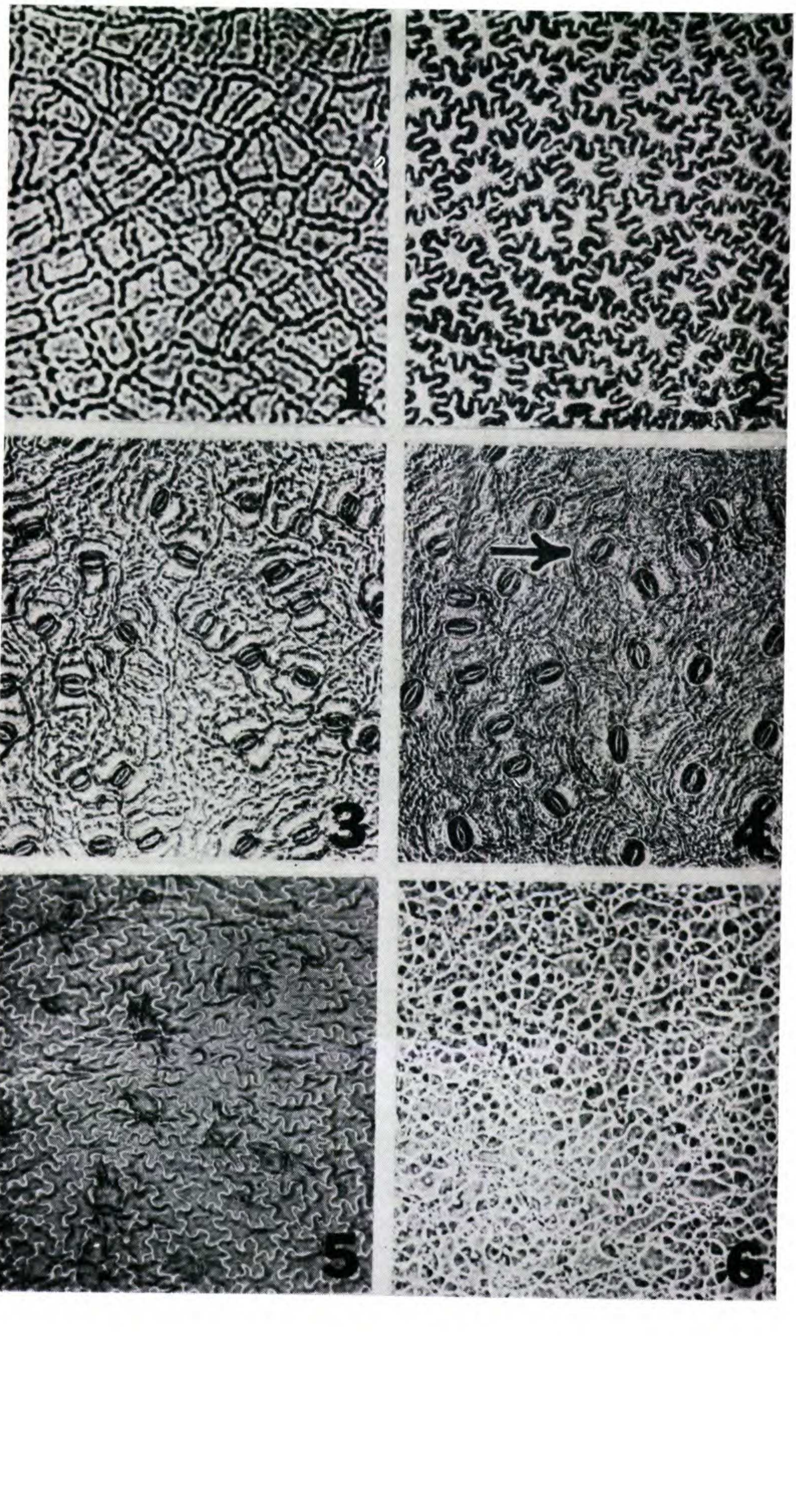
1% solution of phloroglucin in 95% ethyl alcohol and 25% hydrochloric acid (Johansen, 1940), as a specific test for localization of lignified cell walls.

Study of the epidermal imprints and cross sections revealed characteristics which varied from those common to all taxa of *Smilax* studied to those which were unique to single taxa. The interpretations presented below are necessarily based on characteristics of the leaf at the positions where data were obtained, namely one-tenth of the total leaf length beneath the tip for cross sections and one-third of the length for the epidermal imprints. We do not maintain or imply that the characteristics are the same elsewhere. The surface features and gross anatomy of the leaves will be presented separately.

Characteristics of the stomatal cells could be determined from epidermal imprints made from untreated dried material. However, imprints made from FAA preserved or NaOH reconstituted specimens were required for most characteristics of the other epidermal cells.

The appearance of cells at the marginal and veinal areas is that of parallelly aligned, rectangularly elongated structures with striated surfaces and thick cell walls. On the upper epidermis, the interveinal cell type generally changes abruptly to the marginal type; whereas, on the lower epidermis, the change is gradual. The number of rows of distinctly marginal cells of the upper epidermis ranges from 8 to 14.

Typically the number of rows of rectangularly elongated cells at the midvein is approximately three times greater on the lower epidermis than on the upper. Although there are exceptions within some taxa, the cells of the interveinal areas change abruptly at all veinal areas. At the midvein, the number of rows of rectangularly elongated upper epidermal cells is 2 to 11; at the lateral veins, 2 to 8; and at secondary veins, 0 to 5. Generally, there are no rectangularly elongated cells above the venule reticulum on the upper or lower epidermis. The number of rows of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rectangularly elongated lower epidermal cells at the midvein is 5 to 50; at the lateral veins, 0 to 11; and at the secondary veins, 0 to 3.

Although cells of interveinal regions vary considerably from species to species, there are some characteristics which are common to all. All cells of interveinal regions are irregularly aligned (Figs. 1, 2). Generally, undulated cell walls (Fig. 2) are thinner than straight cell walls (Fig. 1). At corresponding locations on the upper and lower epidermises, the cells are essentially the same size. Adjacent cells at any given interveinal location, with the exception of *S. smallii*, are essentially the same size.

The stomatal-subsidiary cell relationship of all species in the terminology of Metcalf and Chalk (1950) is paracytic (Fig. 3). The distal ends of the stomates in all species are aligned at various angles with respect to the longitudinal axis of the leaf (Figs. 3, 4). Differences in microscopic counts and measurements of the stomates, without quantitative measurements and statistical treatment, are usually not sufficiently evident to a cursory examination with the microscope to allow quick identification of taxa. The mean stomatal frequency in a field of vision of 0.093 mm^2 is 3.6 to 5.6; the mean stomatal length, 35.3 to 52.9 microns; and the mean stomatal width, 17.9 to 28.6 microns.

Many differences in epidermal characteristics of the various kinds of *Smilax* studied were revealed by critical examination of the imprints. Those individually unique to a given taxon will be presented first.

The unique characters are as follows:

S. auriculata: One of the two subsidiary cells is crescent

FIGURES 1-6. Epidermal imprints, × 125.

Fig. 1. *S. auriculata*, upper. Note indistinct features due to rough surface cell walls. Fig. 2. *S. glauca*, upper. Fig. 3. *S. bona-nox*, lower, showing large paracytic type subsidiary cells. Fig. 4. *S. auriculata*, lower. Note irregular outer surface of cell walls. Arrow at crescent shaped subsidiary cell. Fig. 5. *S. rotundifolia*, lower, showing striated subsidiary cells. Fig. 6. *S. smallii*, upper, showing large cells separated from each other by a row of much smaller cells.


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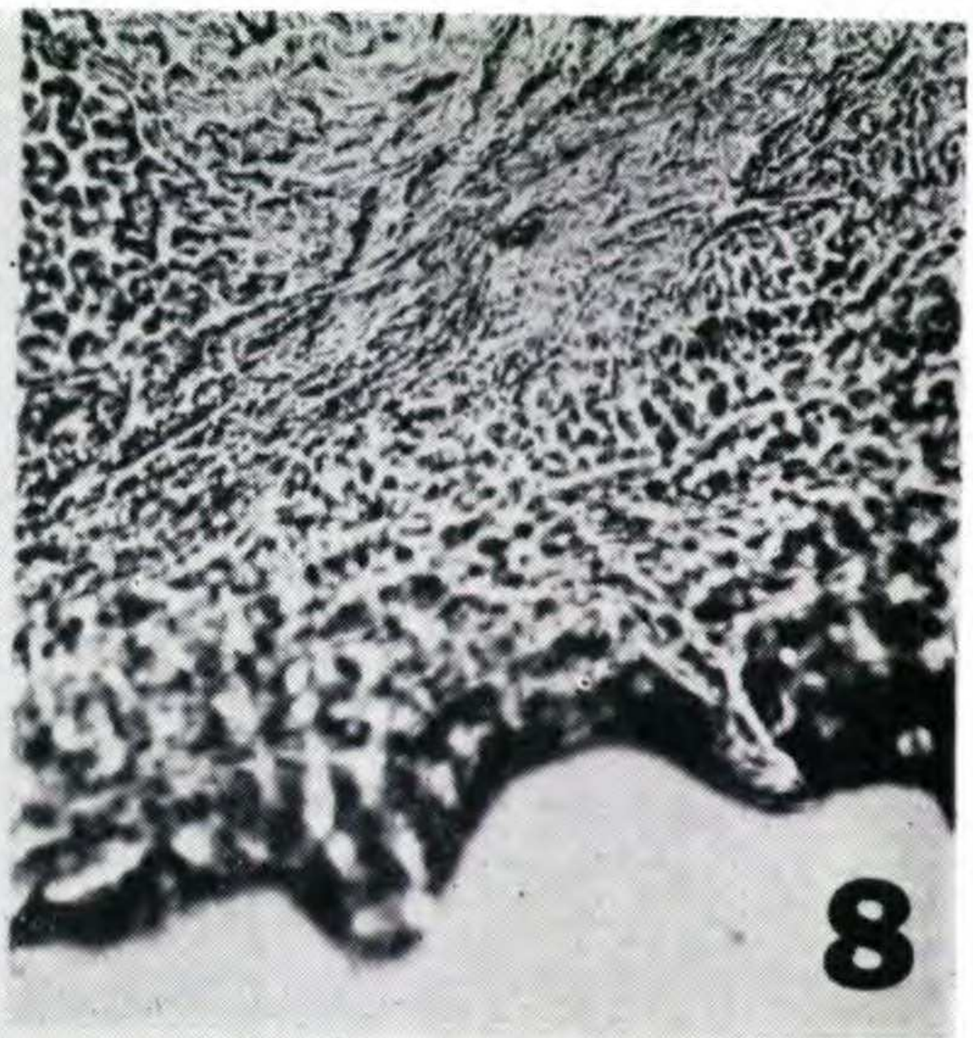
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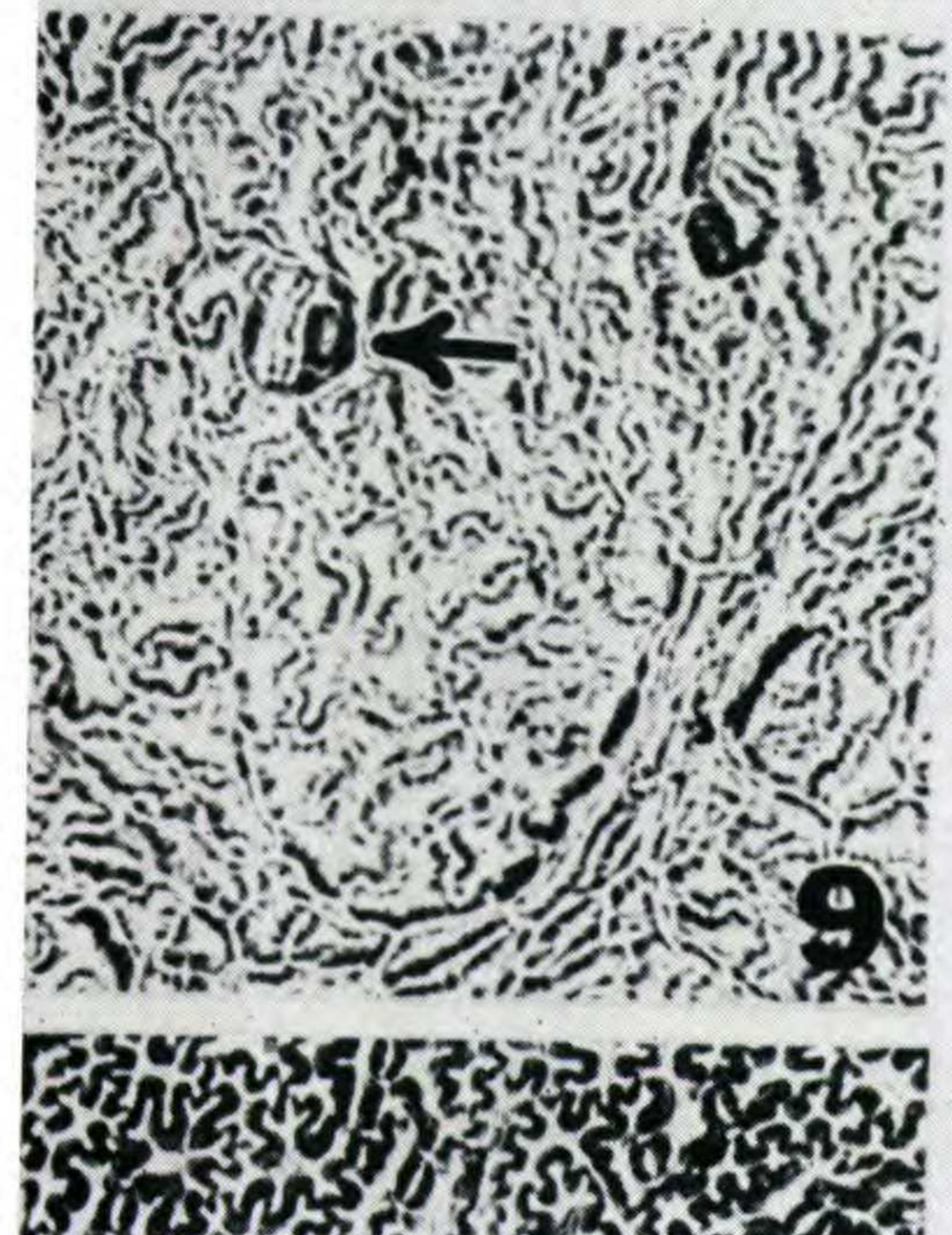
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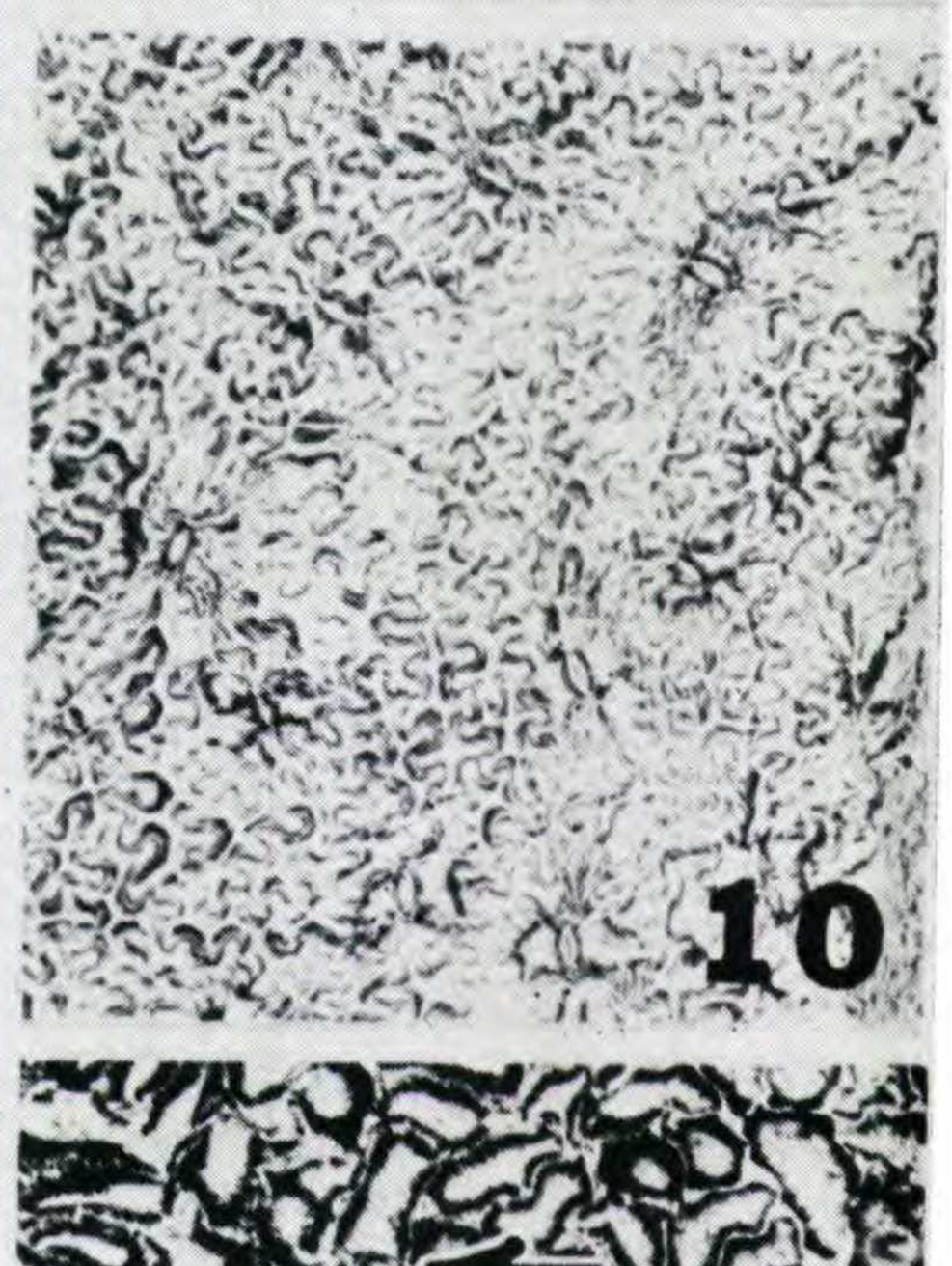
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shaped (Fig. 4), rarely otherwise. In other taxa these are similar in shape to other interveinal cells (Fig. 5).

S. smallii: Interveinal areas of the upper epidermis have large cells separated from each other by a row of much smaller cells, about one-sixth the size of the former (Fig. 6). Also, this is the only taxon having a combination of lateral cell walls of the upper epidermis with straight configuration and of the lower with undulate (Fig. 7).

S. tamnoides: Rectangular cells are present and striated at the venule reticulum on the upper and lower epidermis.

S. tamnoides var. *hispida:* Some marginal rectangular cells converge into minute marginal denticulations (Fig. 8).

S. walteri: Rectangular cells are present at the venule reticulum only on the lower epidermis. Also, this taxon is the only one with both upper (Fig. 9) and lower epidermal cells having a curved configuration, and with the exposed surface of the upper epidermal cells appearing depressed in the middle (Fig. 9).

Characteristics which are common to only two or a few kinds of *Smilax* are several:

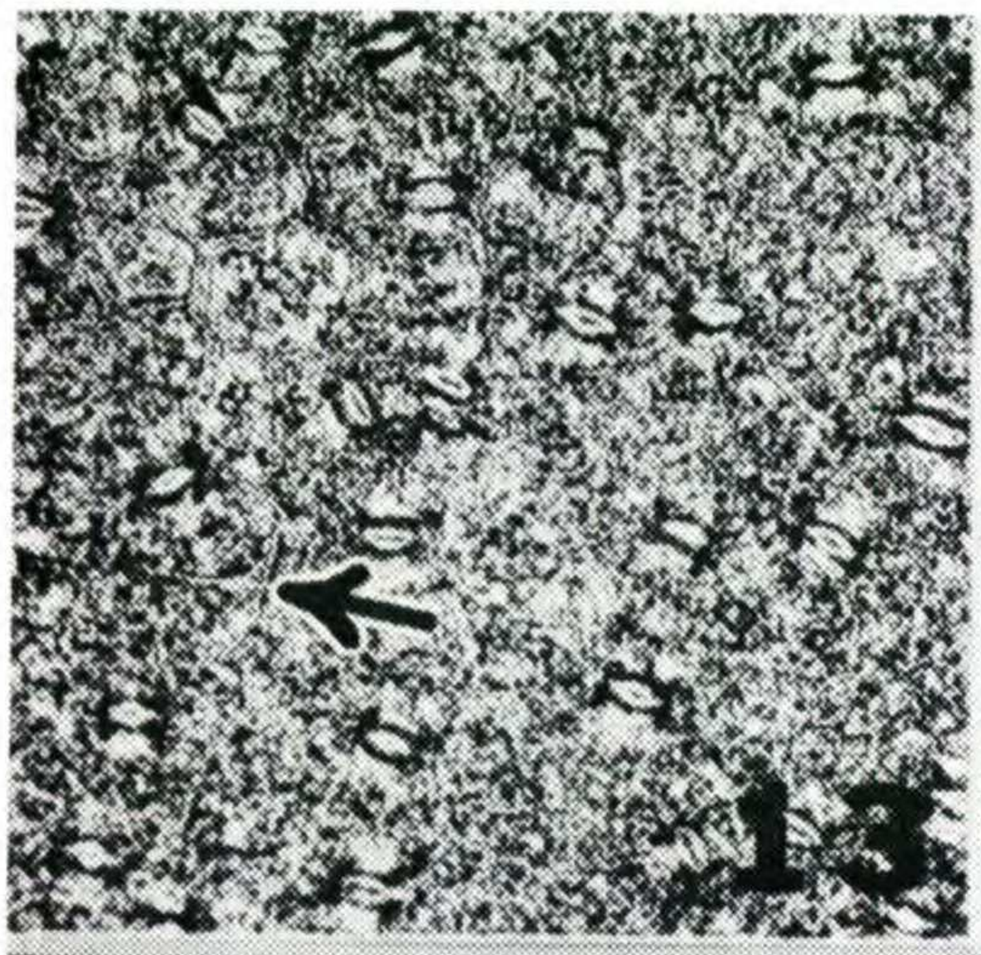
Stomates regularly present on the upper epidermis — *S. rotundifolia*, *S. walteri* (Fig. 9), frequently in *S. tamnoides*. These findings are essentially the same as those reported by Coker (1944) except that no stomates were found in *S. tamnoides*.

Surface of lower epidermal cells quite irregular — *S. auriculata* (Fig. 4), *S. laurifolia*.

Subsidiary cells larger than interveinal cells — *S. bona-nox* (Fig. 3), *S. smallii* (Fig. 7), *S. walteri*.

Surface of most subsidiary cells striated — *S. rotundifolia* (Fig. 5), *S. tamnoides* (Figs. 10, 11), *S. walteri*. A few striations were observed in *S. smallii* and in the ovate leaf form of *S. bona-nox*.

Fig. 7. *S. smallii*, lower. Arrow at undulate cell wall. Fig. 8. *S. tamnoides* var. *hispida*, upper. Fig. 9. *S. walteri*, upper. Arrow at stomate. Fig. 10. *S. tamnoides* var. *hispida*, lower. Fig. 11. *S. tamnoides* var. *tamnoides*, lower. Fig. 12. *S. glauca*, lower. Arrow at stomate.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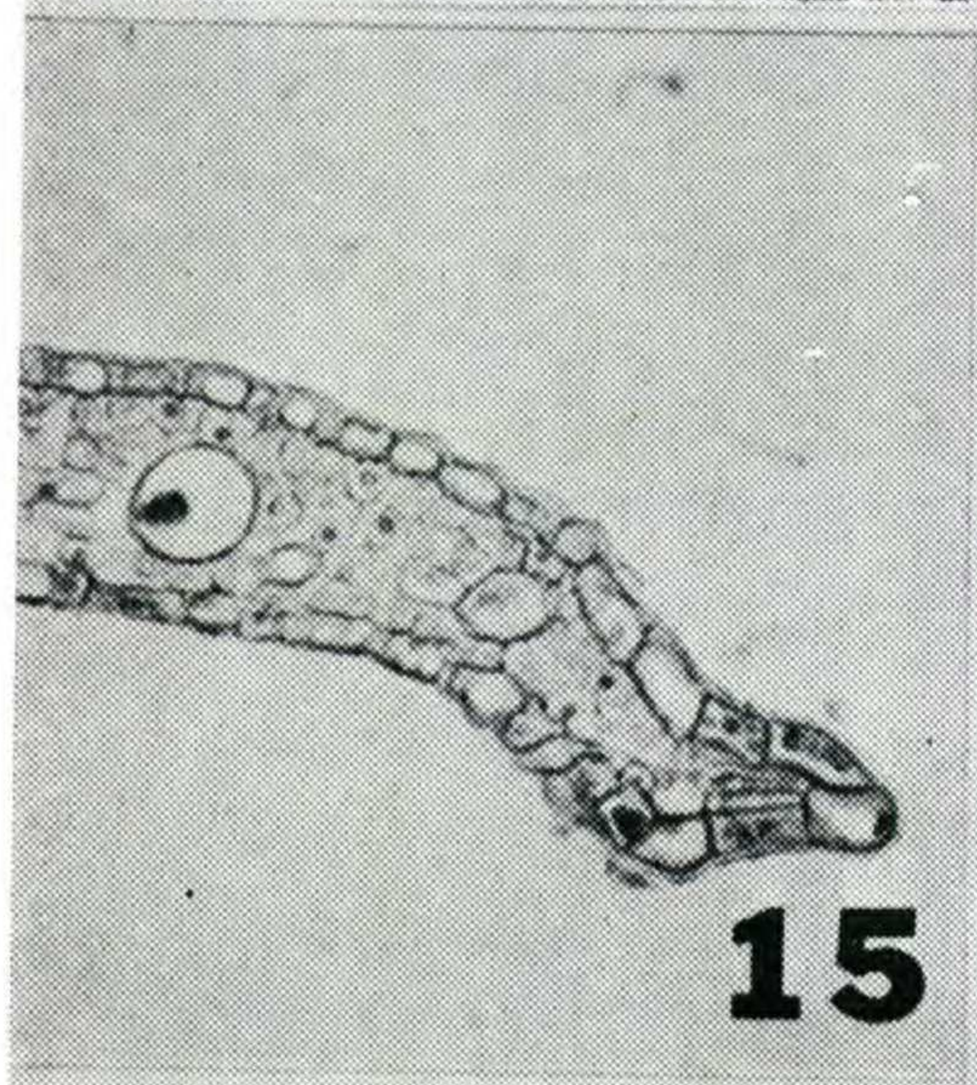
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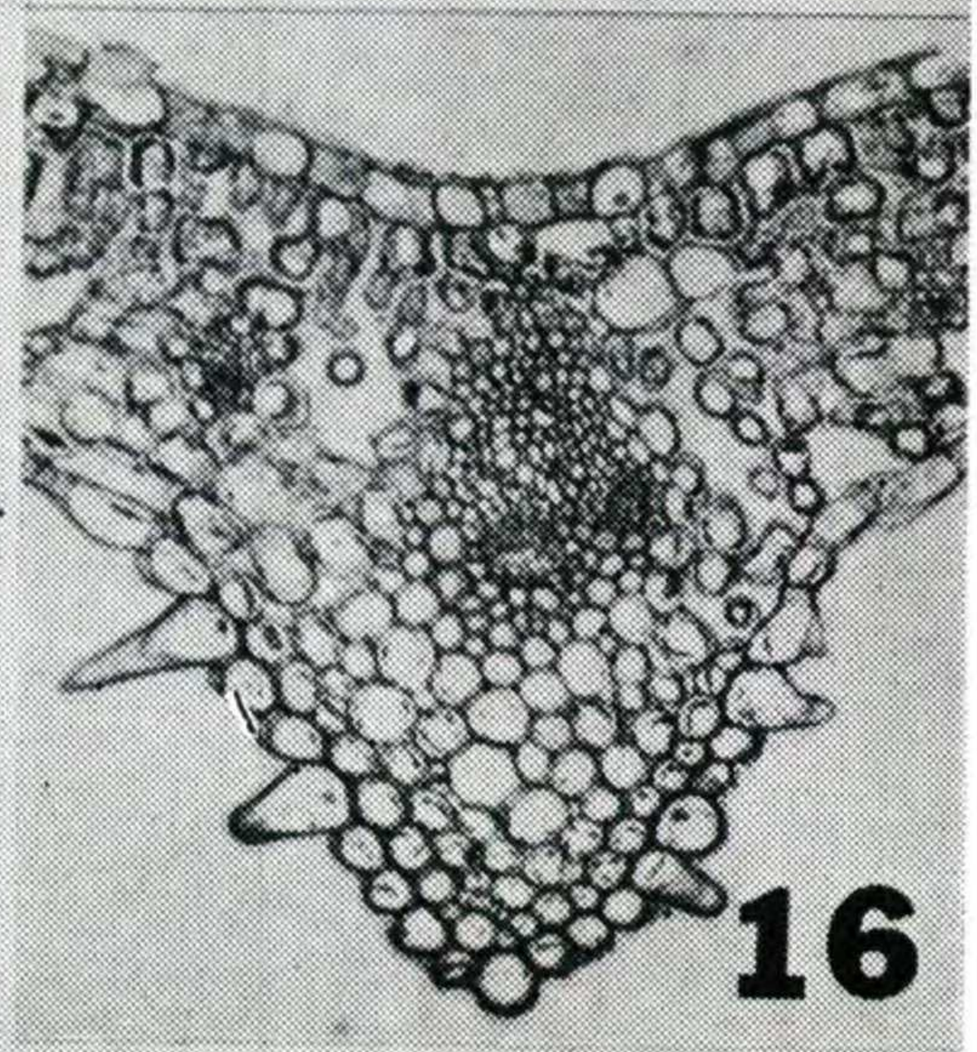
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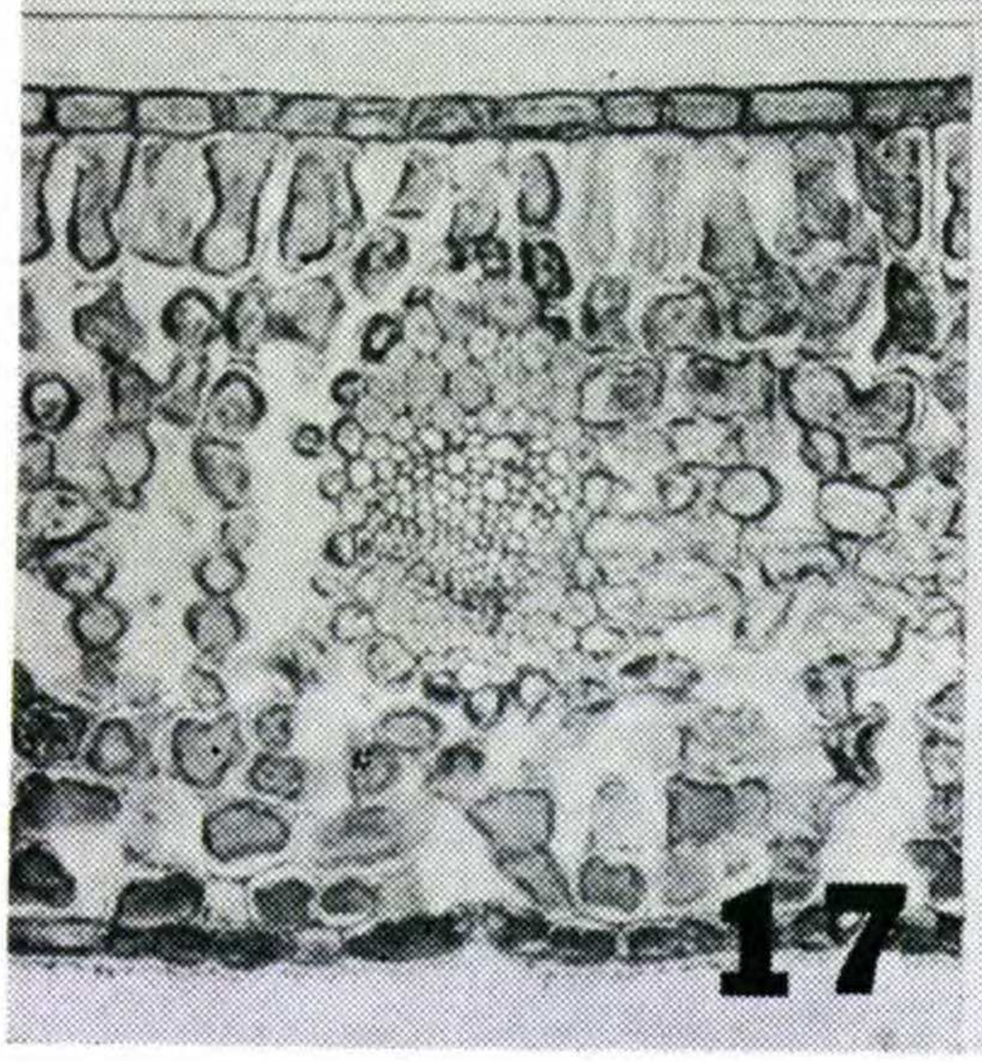
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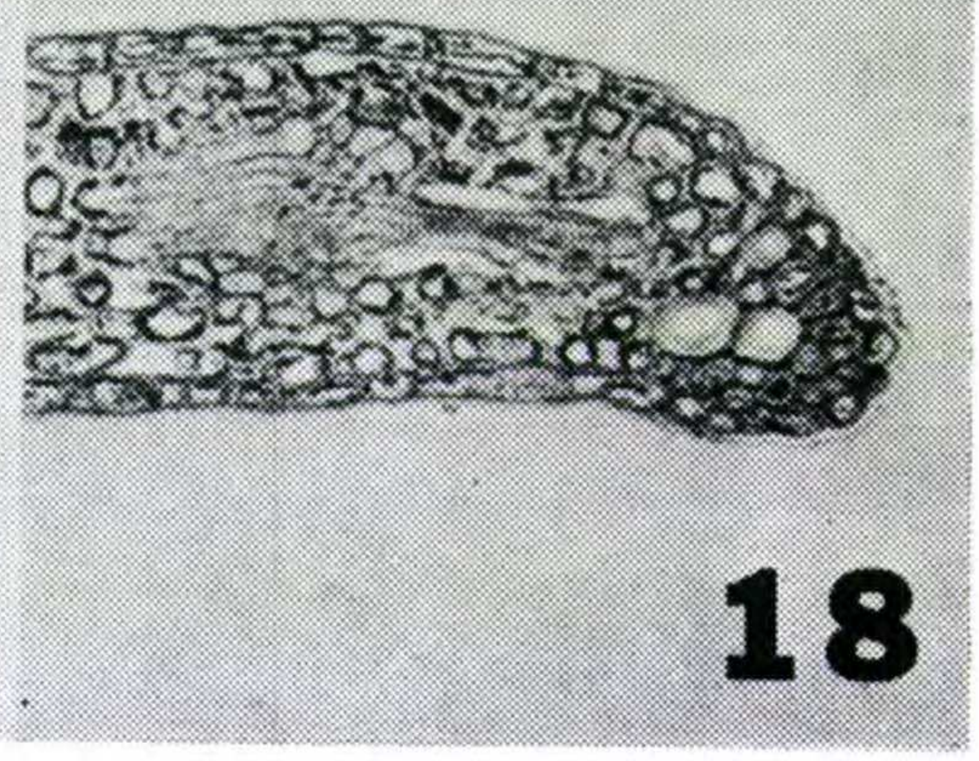
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Rows of marginal rectangular cells on the upper epidermis 10 or less — *S. glauca*, *S. rotundifolia*; more than 20 — *S. tamnoides*, *S. walteri*; 11-18 — the other four taxa.

Lateral cell walls usually both thick and having a configuration that is straight in the upper epidermis — *S. auriculata* (Fig. 1); *S. smallii* (Fig. 6); usually in *S. bona-nox*; and sometimes in *S. laurifolia*, *S. tamnoides* var. *tamnoides*. The configuration is curved in *S. walteri* (Fig. 9) and in three collections of *S. laurifolia* from mesophytic habitats. In all other instances (*S. glauca*, *S. rotundifolia*, six collections of *S. bona-nox*, and two of *S. tamnoides* var. *tamnoides*) the lateral cell walls are thin and have an undulate configuration (Fig. 2). The specimens of *S. bona-nox* having straight walls in the upper epidermis are all from maritime sandy habitats, whereas those with undulate walls are from the Upper Coastal Plain, Piedmont, and Blue Ridge. Configurations of the lateral cell walls of the lower epidermis are the same as for the upper except in *S. glauca* (Fig. 12) which has curved; *S. smallii* (Fig. 7), undulate; *S. laurifolia* (Fig. 13), always straight; *S. tamnoides* (Figs. 10, 11), always undulate; and *S. bona-nox* in which 6 of the 15 collections are undulate as compared to straight.

Exposed cell surfaces of the upper epidermis rough — *S. laurifolia* (Fig. 14); *S. auriculata* (Fig. 1); sometimes rough in *S. bona-nox*, *S. tamnoides*. They are smooth in other instances for the last two species and in the other taxa (Fig. 6).

Exposed surfaces of the lower epidermal cells smooth (Fig. 11) — all taxa except *S. auriculata* and *S. laurifolia*.

Fig. 13. *S. laurifolia*, lower. Note indistinct features due to ridges on surface walls. Arrow at straight cell wall. Fig. 14. *S. laurifolia*, upper. Note indistinct features due to rough surface walls.

Fig. 15. *S. tamnoides* var. *hispida*, showing duct and marginal denticulation. Fig. 16. *S. glauca*, showing papillose lower epidermal cells and prominent midrib. Fig. 17. *S. laurifolia*, showing large first row of palisade and lack of protruding midrib. Fig. 18. *S. bona-nox*, showing sclerenchyma cells in leaf margin.

In the latter species the surface is so deeply ridged as to render imprints opaque when viewed with the naked eye. The ridges obscure cell wall outlines on imprints from *S. laurifolia* (Fig. 13) to the extent that under microscopic examination maximum illumination is usually necessary to discern the configuration of the lateral cell walls.

The ratio of the number of rows of rectangular cells at the midvein of the lower epidermis to the number at the margin is lowest in *S. laurifolia* and *S. smallii* (0.3-0.7) and highest in *S. bona-nox* (0.6-2.0). The ratios for these and the other taxa are given by Yates (1968).

The means for stomatal frequency per 0.093 mm^2, and stomatal length and width measurements in microns are presented in Table 1. Means connected by one or more of the lines beneath are not significantly different at the levels indicated. Means not connected are statistically significant. Each species is indicated by the first letter of the specific epithet, and where applicable, the second letter designating the leaf form or varietal epithet. The leaf forms of *S. bona-nox* are indicated by Ba, Bo, and Bc. The varieties of *S. tamnoides* are designated by Th and Tt.

Sections stained with safranin-aniline blue proved to be more satisfactory for studies of the cuticle, and those stained with Conat's quadruple combination enabled a more discrete examination of the vascular system. Both staining procedures were equally revealing of other aspects of the gross anatomy.

The surface of the cuticle in all species is smooth on the adaxial side and irregularly ridged on the abaxial side. In most species, it is thicker on the adaxial surface than on the abaxial surface and thicker still at the margins. At the leaf margins of most species, rib-like cuticular projections extend inwardly between the radial walls of the epidermal cells.

The continuity of the uniserate, compactly arranged epidermis is interrupted only by the presence of stomates and the aforementioned cuticular projections. Stomates are more distinct and more frequent in the lower epidermis of

Table 1 Mean values of stomatal frequency per 0.093 mm² (I) and stomatal length (II) and width (III) in microns, and the significance of the difference between means[1] for eleven kinds of *Smilax*.[2]

I

Tt	R	Th	Bo	A	Bc	L	S	W	Ba	G
3.61	4.33	4.45	4.46	4.47	4.54	4.71	4.91	4.91	5.23	5.61

II

G	R	Th	Tt	W	S	Bo	Bc	Ba	A	L
35.3	39.0	40.8	43.0	45.4	47.9	49.9	50.3	52.1	52.9	55.3

III

G	W	Tt	R	Th	L	S	Ba	Bc	Bo	A
17.9	19.9	21.7	21.8	22.5	25.3	26.5	27.9	28.5	28.6	28.6

[1]Not significant at 1% level ———, at the 5% level - - - - - -. Explanation in text.

[2]Abbreviations indicate kinds of *Smilax* and are explained in the text.

all species than in the upper epidermis, where in most species they are completely absent. As seen in cross section, the epidermal cells adjacent to the guard cells do not differ markedly from other epidermal cells. In all taxa, the stomates are slightly sunken below, or are level with, the surface of the other epidermal cells. Outer and inner stomatal ledges as described by Esau (1953) project into the stomatal cavity.

At corresponding locations on the adaxial and abaxial sides of the leaf, the epidermises are of approximately equal thicknesses. The cells of the epidermis of most species are rectangular and thick-walled, gradually changing into circular cells with still thicker walls at the leaf margins and veinal areas. At the midrib, more cells are circular and thicker walled in the lower epidermis than in the upper epidermis; whereas, at the major lateral veins, as many cells possess these characteristics in the upper epidermis as in the lower epidermis. The epidermal cells contain no well developed plastids.

The mesophyll is differentiated into palisade parenchyma on the abaxial side. The palisade parenchyma is compactly arranged and may be composed of from one to three layers of cells. The spongy parenchyma is loosely arranged and may be composed of from three to ten layers of cells. At the margins and veinal areas, the mesophyll cells are typically thicker walled and more compactly arranged than mesophyll cells of interveinal regions. They may differ in size, shape, lignification of walls, and affinity for safranin.

The veinal system is composed of collateral vascular bundles with the largest vein being directed along the median longitudinal axis of the leaf. Sclerenchyma encloses the midvein and the major lateral veins. Surrounding the sclerenchyma is a bundle sheath composed of thin-walled parenchymatous cells with a chloroplast complement similar to the mesophyll. As previously stated, the tissue above and below major veins may differ from the mesophyll of interveinal areas.

Randomly scattered throughout the mesophyll of the leaf

cross sections are ducts (Fig. 15) which appear to be single celled and seem either to be void of contents or to contain compact clusters of elongated crystals. The ducts may have thick or thin walls. They may run longitudinally or at an angle.

Many differences in the gross anatomy of leaves of the kinds of *Smilax* studied were revealed by critical examination of the cross sections. Those individually unique to a given taxon will be presented first.

The unique characters are as follows:

S. glauca: Many lower epidermal cells are papillose and most outer walls convex (Fig. 16). The papillae are the most conspicuous in leaves of plants from the more moist habitats and least evident in those from drier habitats. These cells are flattened or rounded and lack papillae in the other species. Coker (1944) reports papillae for his "roughened" form of *S. glauca* but not for his "glabrous" form. In making no reference to papillae in the other taxa he studied, the impression is left that none were found. In our studies also, the ratio of the thicknesses of the cuticle at the margin of the blade to the thickness of the adaxial cuticle at an interveinal area 1.5 mm from the margin is always less than 2:1. In all other taxa it is greater than 2:1. In addition, the ratio of the thickness of the cuticle of the upper epidermis to that of the lower is 4:1 or greater, whereas in other taxa the ratio is about 2:1 or less.

S. tamnoides: Uppermost palisade parenchyma cells are not as thick or are as thick as the upper epidermal cells (Fig. 15), whereas they are thicker than the upper epidermal cells in all other taxa. Also mesophyll cells at the leaf margin differ from those of interveinal mesophyll by being irregularly shaped and small.

S. tamnoides var. *hispida:* Marginal denticulations are discernible from serial cross sections (Fig. 15).

S. laurifolia: The leaf thickness (about 670 microns) at interveinal areas near the midvein is greater (Fig. 17) than for the other species in which the thickness is usually between 330-370 microns (e.g., Figs. 15, 16, 18). Upper-

most palisade parenchyma cells are more than 2× as thick as upper epidermal cells (Fig. 17). These palisade cells may be nearly this large in *S. rotundifolia*. In all other taxa they are smaller. Also, *S. laurifolia* is the only taxon in which the cuticle on the lower epidermis is thicker than on the upper.

S. bona-nox: More than 15 sclerid cells are in the leaf margin (Fig. 18). Other taxa have none or only one or two such cells.

S. auriculata: Upper epidermal cells are seven or more microns thicker than the lower. In other taxa they are about the same thickness.

Gross anatomical characteristics which are common to only two or a few kinds of *Smilax* are:

Rib-like cuticular projections between marginal epidermal cells — *S. bona-nox*, *S. laurifolia*, *S. smallii*, and *S. walteri*; often in *S. auriculata* and *S. tamnoides*.

Some cell walls adjacent to sclerid cells around veins lignified, the opposite walls not — *S. bona-nox*, *S. tamnoides*.

More sclerenchyma cells on the adaxial side of the major veins than on the abaxial — *S. auriculata*, *S. laurifolia*. Circular cells in the mesophyll at the leaf margin differing from cells of the interveinal mesophyll — *S. laurifolia* and *S. smallii* have smaller circular cells; *S. glauca* has circular cells but they are as large as adjacent interveinal cells.

Conspicuously enlarged cells below the midvein — *S. glauca*; occasionally in *S. rotundifolia* and *S. smallii*.

Midrib generally not protruding — *S. laurifolia* (Fig. 17); barely protruding in *S. smallii*. *Smilax glauca* possesses a most prominent, often ridged, midrib (Fig. 16). The midribs of the other taxa are prominent but are more rounded than in *S. glauca*.

Although as indicated above there are differences between cross sections of *S. rotundifolia*, *S. smallii*, and *S. tamnoides*, the general aspect of sections of the species are quite similar.

Differences between some species occur in the safranin stained protoplast of the mesophyll and epidermis. The

protoplast of the mesophyll of *S. laurifolia* and *S. walteri* is so abundantly stained that the sections are obviously darkened. This protoplast is stained in some of the other species, but there is no gross effect as in the above two species. Except for the two species, staining characteristics are not gross in nature and are not described here. They are described by Yates (1968).

A few other minor differences in gross anatomical characters between taxa are given by Yates (1968).

The results of the present study differ in several respects with the earlier leaf anatomical investigations of Caponetti and Quimby (1956). They report no papillae on the midrib of the lower epidermis. We observed some on the collections from mesophytic habitats. Their failure to find papillae may be due to their collections having been from xerophytic environments only. Also, neither of the staining techniques used in this study reveals the presence of lignin which they reportedly detected in the mesophyll cells abaxial to the midvein of *S. glauca*. No explanation for these different observations seems possible from the information available. Their assumption that the extension of the sclerenchyma surrounding the midvein to the epidermis is characteristic of *S. auriculata* is the exception rather than the rule for specimens analyzed in the present study. Finally, *S. bona-nox* is not the only species, as they state, with the cells surrounding the sclerenchyma of the midvein having thick lignified inner walls and thin non-lignified outer walls. These peculiar cells are also frequently present in *S. tamnoides*. Because of the variation in the taxa involved, it is quite possible that the observations of Caponetti and Quimby were limited to too few specimens to detect the variability.

The present study has also revealed the structural nature of the marginal ribs indicated by Duncan (1967) for *S. bona-nox*, *S. auriculata*, and *S. laurifolia*. Externally these ribs often seem identical to veins but were not labeled as such because no veinal connections with nearby veins were observed. In *S. bona-nox* the rib is largely due to 15 or

more rows of sclerenchyma cells. In *S. auriculata* the rib seems due to the thick cuticle and compactly arranged subepidermal cells which also often has one or two sclerenchyma cells. In *S. laurifolia* a thick cuticle at the margin and compact subepidermal cells probably provide the rigidity to form a rib when the leaf is dried. Frequently some of the sclerenchyma cells in the leaf margin of *S. bona-nox* are much larger than the others and look like vessels. However, since our external and internal studies revealed no connection between the ribs and adjacent veins nor any phloem elements or bundle sheaths, we believe that the term "rib" should be continued in use for all three species.

Analysis of the data of the present study shows that in respect to surface features of the epidermises, the nine taxa differ by unique and/or certain combinations of characters. The same is true for gross anatomical characters. These data, therefore, support the species concept in the section Smilax as interpreted by Duncan (1967). In addition, the two varieties of *S. tamnoides*, as represented by the materials studied, differ in both surface features and gross anatomical characters. However, neither epidermal nor gross anatomical data were found separating the three leaf forms of *S. bona-nox*. Studies should be made in this species to determine whether or not specimens from maritime habitats consistently and exclusively have straight lateral cell wall configuration in the leaf upper epidermis. Such plants may be Coker's (1944) *S. bona-nox* var. *littoralis*. Similar studies should be made in respect to the undulate walls in specimens from other areas. Also, more study is needed to learn why the exposed epidermal cell walls in leaves of *S. walteri* appear depressed in epidermal imprints, yet as seen under microscopic examination of cross sections are not depressed.

Keys presented below emphasize many of the characters most distinctive for the nine taxa and allow identification of specimens on the basis of epidermal or gross anatomical characters. Although the two varieties of *S. tamnoides* may be separated, in the case of the materials we studied,

we hasten to point out that only specimens distinctly one type or the other were examined. Further epidermal and gross anatomical studies are necessary to determine whether the minute denticulations on the leaf margin of var. *hispida* exhibit intergradation as do the leaf forms as described by Duncan (1967). Under a dissecting microscope both varieties appear to have denticulations, but apparently in var. *tamnoides* this is due to uneven crimping and rolling of the especially thin margins of the blade.

Key to Taxa Based on Surface Features of Leaves

1. Upper epidermal cells of interveinal areas having larger cells each being separated from any other by a row of much smaller cells ..... *S. smallii*
1. Upper epidermal cells do not have such an arrangement of larger and smaller cells 2
   2. A crescent shaped subsidiary cell associated with the stomates on the lower epidermis ..... *S. auriculata*
   2. No crescent shaped subsidiary cell associated with the stomates on the lower epidermis 3
      3. Lateral cell wall configuration of lower epidermis curved 4
         4. Lateral cell wall configuration of upper epidermis undulated, stomates absent on upper epidermis ..... *S. glauca*
         4. Lateral cell wall configuration of upper epidermis curved, stomates present on upper epimis ..... *S. walteri*
      3. Lateral cell wall configuration of lower epidermis not curved 5
         5. Lateral cell wall configuration on lower epidermis straight 6
            6. Surface of lower epidermal cells of interveinal areas ridged ..... *S. laurifolia*
            6. Surface of lower epidermal cells of interveinal areas not ridged ..... *S. bona-nox*
         5. Lateral cell wall configuration on lower epidermis undulated 7

7. Lower epidermal cells associated with venule reticulum and striated *(S. tamnoides)* 8
8. Marginal cells converge to form minute marginal dentations ..... *S. tamnoides* var. *hispida*
8. Marginal cells do not converge to form minute marginal dentations ..... *S. tamnoides* var. *tamnoides*

7. Lower epidermal cells associated with venule reticulum not different from those of interveinal areas 9
9. Stomates present on upper epidermis, subsidiary cells about same size as interveinal cells ..... *S. rotundifolia*
9. Stomates absent on upper epidermis, subsidiary cells larger than interveinal cells ..... *S. bona-nox*

Key to Taxa Based on
Gross Anatomical Characters of Leaves

1. Fifteen or more sclerenchyma cells present in margin of leaf ..... *S. bona-nox*
1. Less than four sclerenchyma cells present in margin of leaf 2
2. Large papillose cells present in lower epidermis, ratio of thickness of the cuticle at the leaf margin to the thickness of the cuticle on the upper epidermis at an interveinal area 1.5 mm from the margin always less than 2:1 ..... *S. glauca*
2. Large papillose cells not present in lower epidermis, ratio of the thickness of the cuticle at the leaf margin to the thickness of the cuticle on the upper epidermis at an interveinal area 1.5 mm from the margin always 2:1 or greater 3
3. Uppermost palisade parenchyma cells not as thick as upper epidermal cells *(S. tamnoides)* 4

4. Marginal denticulations on leaf evident in serial cross sections ..... *S. tamnoides* var. *hispida*
4. Marginal denticulations on leaf not seen in serial cross sections ..... *S. tamnoides* var. *tamnoides*

3. Uppermost palisade parenchyma cells thicker than upper epidermal cells 5
5. Uppermost palisade parenchyma cells more than twice as thick as upper epidermal cells, cuticle on the lower epidermis thicker than on the upper ..... *S. laurifolia*
5. Uppermost palisade parenchyma cells not more than twice as thick as upper epidermal cells, cuticle on the lower epidermis as thick as or thinner than on the upper 6
6. More sclerenchyma cells on adaxial side of major veins than on the abaxial side ..... *S. auriculata*
6. As many or fewer sclerenchyma cells on the adaxial side of major veins as on the abaxial side 7
7. Small circular cells present in the mesophyll at the leaf margin ..... *S. smallii*
7. Small circular cells absent in the mesophyll at the leaf margin 8
8. Mesophyll deeply stained with safranin ..... *S. walteri*
8. Mesophylll scarcely stained with safranin ..... *S. rotundifolia*

We pointed out earlier that identification of sterile specimens to taxa is often difficult. Epidermal and gross anatomical characteristics revealed by this study help alleviate this problem. Gross anatomical characteristics are especially useful in the case of collections of *S. bona-nox* which, partly because of polymorphic leaf shapes, are often incorrectly identified as *S. rotundifolia*, *S. tamnoides*, *S. auriculata*, or

S. smallii. Even though the character of the marginal rib of *S. bona-nox* leaves can give positive identification, the diagnostic value of the rib is lessened because the thin margins which occur in some of the other taxa may be enrolled and appear enlarged, especially on dried specimens. Since our studies have revealed the margin to contain many sclerenchyma cells, positive identification of both dried and fresh specimens of *S. bona-nox* may be accomplished from freehand leaf cross sections of approximately 5 mm thickness by staining with phloroglucin as described earlier. Within five minutes this stain will impart a purple-reddish color to lignified structures, and examination with a 10× lens will reveal the presence or absence of the unique abundance of marginal sclerenchyma cells for *S. bona-nox.*

As is evidenced from the keys and other data presented earlier, all taxa possess both epidermal and gross anatomical characteristics that are distinguishing. However, except for *S. bona-nox* which is discussed above, epidermal imprints are more practical for identification purposes than are cross sections because of the time element and equipment involved. Imprint characteristics useful in checking identifications of specimens in some of the situations in which taxa are easily confused are pointed out below.

Some specimens of *S. smallii* are similar to those of *S. auriculata* and *S. laurifolia* but may be readily distinguished from them by a unique upper epidermis where each larger cell is separated from any other by a row of smaller cells approximately one-sixth the size of the former.

Smilax auriculata is easily distinguished from similar forms of *S. tamnoides* and *S. laurifolia* by a crescent shaped subsidiary cell flanking each stomate.

When specimens of *S. glauca* are dried, the glaucous nature of the underside of the leaf is often not apparent and the specimens are easily confused with those of *S. rotundifolia.* They may be identified by the absence of stomates in the upper epidermis of the former and by their presence in the latter.

Some leaf forms of *S. tamnoides* and of *S. rotundifolia* are similar. They may be identified by rectangular cells

above the venule reticulum of both upper and lower epidermises in the former, and by a higher ratio (2.5-5.0/0.8-1.0) of rows of rectangular cells beneath the midvein to rows at the leaf margin in the latter. *Smilax walteri*, which is often similar to *S. rotundifolia*, is distinguished from the latter by the same ratios of rectangular cells. In addition, *S. walteri* has curved lateral cell walls on both epidermises while in *S. rotundifolia* they are undulate.

Phylogenetic relationships between the Southeastern United States species of Smilax, excepting *S. pumila* which was not studied, seem more evident after an analysis of the data obtained in this study. A detailed presentment of presumed relationships does not seem justified here, but a summary of the more important aspects does. In coming to our conclusions, data published previously were considered as well as the data we obtained.

Smilax laurifolia is closely related to *S. auriculata* and *S. smallii* only, and these latter two species are much more closely related to *S. laurifolia* than to any other species. This is compatible with Coker's (1944) data on underground parts. Because of these strong relationships the three species should be considered as a single group even though *S. laurifolia* and *S. smallii* are not strongly related.

A second group of species consists of the closely related *S. bona-nox*, *S. tamnoides*, and *S. rotundifolia*. This group is loosely tied to the first by two similarities that *S. bona-nox* has with *S. auriculata* only and by one character it has with *S. smallii* only. Also, *S. tamnoides* has one character in common with *S. auriculata* only.

The remaining two species involved in this study, *S. walteri* and *S. glauca*, are not sufficiently related to be considered as a third group. They stand instead as single taxa with relatively weak relationships to each other and to certain taxa in the second group.

Smilax walteri is more closely related to *S. rotundifolia* than any other species. This is also supported by Coker's (1944) data on underground parts. *Smilax walteri* also has a character in common with *S. tamnoides* only. However, *S. walteri* has fewer ties to and more differences from the

members of the *S. bona-nox* — *S. tamnoides* — *S. rotundifolia* complex, than they to each other, and best should not be included with them.

The several unique characteristics of *S. glauca* place it considerably apart from the other species. It is more closely allied to *S. rotundifolia* and *S. walteri* than any other species and therefore should be placed nearest them in any phylogenetic scheme.

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LITERATURE CITED

ARBER, AGNES. 1920. Tendrils of *Smilax*. Bot. Gaz. **69:** 438-442.

CAPONETTI, JAMES D. and MAYNARD W. QUIMBY. 1956. The comparative anatomy of certain species of *Smilax*. J. Amer. Pharm. Ass. Sci. Ed. **45**(10): 691-696.

COKER, W. C. 1944. The woody smilaxes of the United States. J. of Elisha Mitchell Sci. Soc. **60:** 27-69.

DUNCAN, W. H. 1967. Woody vines of the southeastern states. Sida **3:** 1-78.

ESAU, KATHERINE. 1953. Plant anatomy. John Wiley & Sons, Inc., New York. 753 p.

FERNALD, M. L. 1944. Overlooked species, transfers, and novelties in the flora of eastern North America. Rhodora **46:** 1-28, 32-60.

JOHANSEN, D. A. 1940. Plant microtechnique. McGraw-Hill, New York. 523 p.

METCALFE, C. R. and L. CHALK. 1950. Anatomy of the dicotyledons. Clarendon Press, Oxford. Vol. 1.

MORONG, THOMAS. 1894. The Smilacaceae of North and Central America. Bull. Torr. Bot. Club **21:** 419-443.

SINCLAIR, C. B., and D. B. DUNN. 1961. Surface printing of plant leaves for phylogenetic studies. Stain Tech. **36:** 299-309.

SMALL, J. K. 1933. Manual of the southeastern flora. The autnor, New York. 1554 p.

STEEL, ROBERT G. D. and JAMES H. TORRIE. 1960. Principles and procedures of statistics. McGraw-Hill, New York. 481 p.

YATES, IDA E. 1968. Comparative studies of *Smilax*, Section Eusmilax, of the Southeastern United States. Masters Thesis. University of Georgia, Athens, Georgia.